Rachel's Lab Notebook

August 3rd

Objectives:

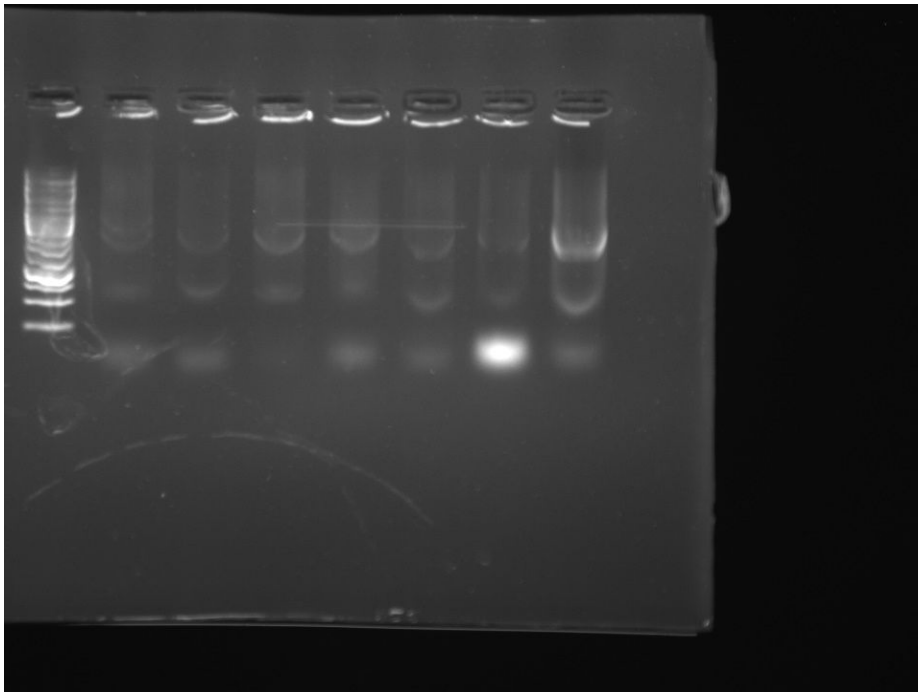
- Restriction digest for chromoproteins, terminators, and promoters (5F, 24D, 19E, 6C, 6K, 16E, 3F)
- Gel electrophoresis to ensure all parts were properly digested

Procedures:

Plasmid	Amount Water (in uL)	Amount DNA (in uL)	Buffer Used (5uL)	Enzyme used (1uL of each)
5F	31	12.6	3.1	X & P
24D	34.97	8.03	3.1	X & P
19E	16.58	26.42	3.1	X & P
6C	18.10	24.89	3.1	X & P
6K	28.4	14.6	3.1	X & P
16E	24.76	17.24	2.1	E & S
3F	26.57	16.43	2.1	E & S

Results:

- All plasmids were digested successfully (from left to right: 1kb ladder, 5F, 24D, 19E, 6C, 6K, 16E, 3F)



August 4th

Objectives:

- Miniprep: amp bb (4N), chlor bb (13L), terminator(1B), yellow luciferase
- Restriction digest: amp bb, chlor bb

Procedures:

- Standard Biomiga miniprep procedure
- Restriction Digest Procedure:

Undigested Plasmid	Amount water (in uL)	Amount DNA (in uL)	Buffer Used (5 uL)	Enzyme used (1 uL of each)
Chloramphenicol backbone	16.89 uL	26.11 uL	2.1	EcoR1 Pst1
Ampicillin backbone	25.46	17.54	2.1	EcoR1 Pst1

- Incubated at 37℃ for 1 hour
- Incubated at 80℃ for 20 minutes

Results:

Miniprep Results:

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	4N chlor bb - 1	Student	8/4/2015 12:59:00	70.1	ng/µl	1.402	0.754	1.86	3.03	DNA	50
2	4N chlor bb - 2	Student	8/4/2015 13:00:00	76.9	ng/µl	1.538	0.847	1.82	2.66	DNA	50
3	13 L	Student	8/4/2015	114.4	ng/µl	2.287	1.227	1.86	2.52	DNA	50

	ampicillin bb - 1		13:02:00								
4	13 L ampicillin bb - 2	Student	8/4/2015 13:03:00	91.2	ng/µl	1.824	1.023	1.78	2.6	DNA	50
5	1B terminator - 1	Student	8/4/2015 13:05:00	82.5	ng/µl	1.649	0.913	1.81	2.19	DNA	50
6	1B terminator - 2	Student	8/4/2015 13:06:00	67	ng/µl	1.34	0.723	1.85	2.29	DNA	50
7	yellow luciferase	Student	8/4/2015 13:07:00	68.2	ng/µl	1.363	0.738	1.85	2.5	DNA	50

August 5th

Objectives:

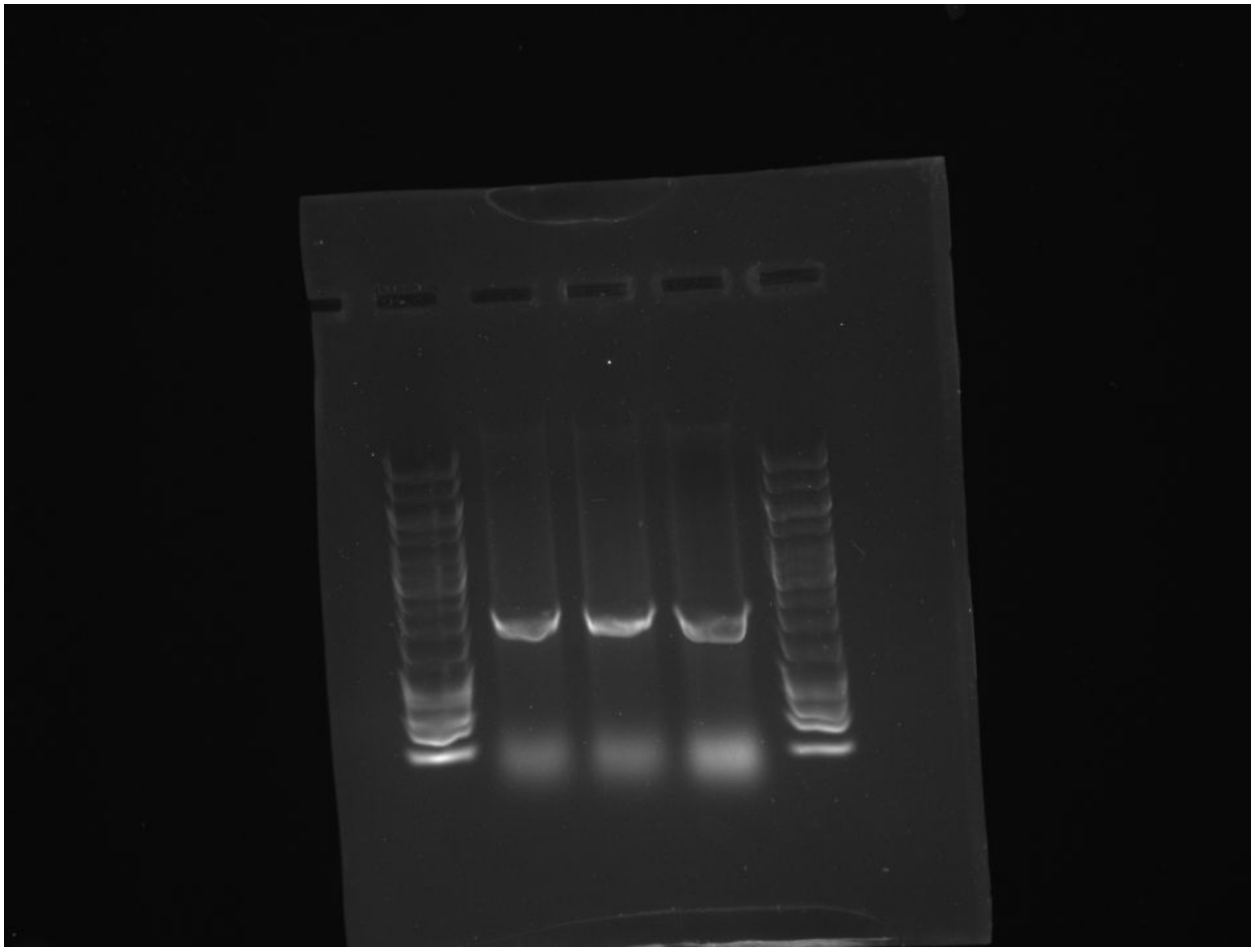
- Gel Extraction of digested backbones from August 4th (amp backbone - 4N; chlor backbone - 13L)
- Conduct research on luciferase/order luciferin
- Continue recording inventory of parts

Procedures:

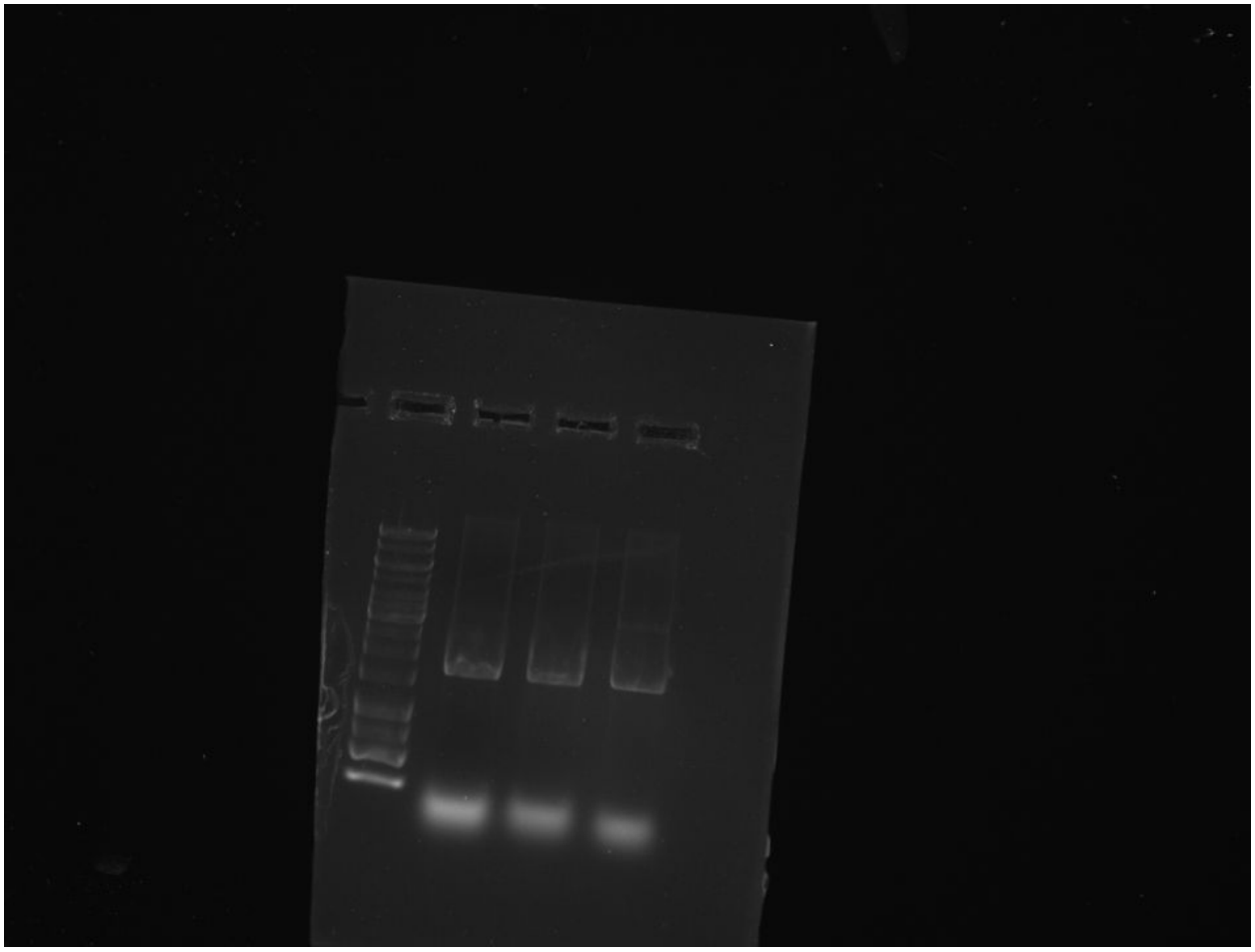
- Add 10 uL of 6x loading dye to 50 uL of amp/chlo backbone
- Load wells of a 0.75% gel as follow:
    - 1kb ruler (10 uL), chlor(20 uL), chlor, chlor, amp(20 uL), amp, amp, 1kb ruler (10uL)
    - Run gel at 75 V for 30 minutes

Results:

Chloramphenicol backbone gel:



Ampicillin backbone gel:



- Restriction digest for backbones seems to have failed, gel extraction not continued

August 6th

Objectives:

- Ann told me yesterday that she wants me to put some reporter genes (like RFP) on Reida's p/rbs_chromoprotein_terminator to troubleshoot why we aren't seeing any color
- Rehydrated a ribosome binding site from the plate kit (3755, 4G, BBa_B0030, rbs)
- Transform rbs
- Inoculated Ann's minipreps (check Ann's notebook to see what parts)
- Prepared parts for sequencing by IDT

Procedures:

- Transformed rbs on chloramphenicol plate
    - 2uL of plasmid, 50 uL of DH5a
    - 5 mins ice, 60 seconds 42°C, 5 mins ice, add 200 uL SOC broth
    - incubate on shaker for two hours

Results:

- For IDT, plasmids to be sequenced must be between 100 - 200 ng/ul and 30-50 ul in volume - I gave them 30 ul of each plasmid (unless there wasn't enough of the plasmid)
- I diluted Y7 (lime chromoprotein preT) and X1 (p/rbs+lime+term)

    - Y7 final concentration: 178.6 ng/ul
    - X1 final concentration: 185.2 ng/ul
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August 7th

Objectives:

- Miniprep for Ann
- Preparing parts for sequencing
- Restriction digest for Ann following Ann's protocol

Procedures:

- Biomiga miniprep Kit
- See Ann's restriction digest math

Results:

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#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	6M lime 1	Student	8/7/2015 13:20:00	197.9	ng/µl	3.959	1.942	2.04	2.28	DNA	50
2	6M lime 2	Student	8/7/2015 13:21:00	238.5	ng/µl	4.77	2.284	2.09	2.33	DNA	50
3	2J yellow luc 1	Student	8/7/2015 13:22:00	329.6	ng/µl	6.592	3.173	2.08	2.26	DNA	50
4	2J yellow luc 2	Student	8/7/2015 13:23:00	294.7	ng/µl	5.894	2.839	2.08	2.29	DNA	50
5	5F ggblue 1	Student	8/7/2015 13:24:00	194.1	ng/µl	3.882	1.879	2.07	2.28	DNA	50
6	5F ggblue 2	Student	8/7/2015 13:25:00	133.4	ng/µl	2.668	1.332	2	1.91	DNA	50
7	16E prbs 1	Student	8/7/2015 13:26:00	280.8	ng/µl	5.616	2.647	2.12	2.28	DNA	50
8	16E prbs 2	Student	8/7/2015 13:26:00	382.6	ng/µl	7.652	3.619	2.11	2.33	DNA	50

August 10th

Objectives:

- Inoculate rbs
- Make more chloramphenicol/arabinose and ampicillin/arabinose plates
- Today we got back our sequencing results from Sangon and our parts were terminator - GFP/CFP - terminator; this means that something must be going wrong with restriction digest or maybe ligation; so I'm no longer trying to add reporter genes to our chromoprotein parts (which is why I stop working with rbs after inoculating and miniprepping it)

Procedures:

- Basic inoculation procedure: 12 mL SOC, 12 mL chloramphenicol
- See plating procedure:

Results:

- N/A

August 11th

Objectives:

- Miniprep rbs

Procedures:

- Biomiga Miniprep Procedure

Results:

	Sample ID	User name	Date and Time	Nucleic Acid Conc.	U	A	A	260/	260/230
	4G - B0030 - rbs_1	Student	8/11/2015 12:00:00	269.3	r	5	2.	2.14	2.3
	4G - B0030 - rbs_2	Student	8/11/2015 12:01:00	227.7	r	4	2.	2.11	2.17

August 12th

Objectives:

- See list of things that Ann left for me to do from August 12th
- Transformed: B56 + Chlbb; Y56 + Chlbb; L56 + Chlbb; PCR: 1 (yellow), 2 (lime), 3 (blue) with 20 uL of added arabinose
- Transformed all above plasmids on plate with 200 uL extra ara

Procedures:

- Biomiga Miniprep Procedure
- Inoculation was done with LB rather than SOC with varying concentrations of arabinose (see Ann's document); the OD 600 was measured every 2 hours until it reached ~0.5, then arabinose was added and inoculation continued overnight.

Results:

- Miniprep results for Ann's inoculations (to be sent to Sangon for sequencing) were low

#	Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	lime 2 IDT	Student	8/12/2015 12:04:00	80.9	ng/µl	1.618	0.86	1.88	2.15	DNA	50

2	blue IDT	Student	8/12/2015 12:05:00	19.5	ng/µl	0.389	0.21	1.85	1.68	DNA	50
3	yellow IDT	Student	8/12/2015 12:06:00	22.9	ng/µl	0.459	0.234	1.96	1.76	DNA	50

- Growth was shown on all plates but no color seen
- Inoculation using Ann's experimental concentrations did not produce color
- Arabinose was added after 6 hours of inoculation (b/c OD numbers were too low)

August 13th

Objectives:

- Inoculate luciferase plasmids (Red, Epic, Green, Yellow) from Fudan plates (August 3)
- Prepare lysozyme solution and lysis buffer for tomorrow

Procedures:

- See the document "Luciferase Expression Protocol" for lysozyme solution/lysis buffer preparation
- Inoculation was done with SOC media - arabinose added to half of the inoculations (there were two trials for each plasmid)

Results:

- N/A

August 14th

Objectives:

- Lyse the luciferase plasmids that were inoculated on August 13th
- I spent the rest of the day at Merck Millipore

Procedures:

- For lysis procedure, see the document "Luciferase Expression Protocol"
- After adding lysozyme the lysed bacteria were left to sit at room temperature for two hours and then stored in the -20 freezer until use (after the weekend)

Results:

- N/A

August 17th

Objectives:

- Make a stock solution of d-luciferin and the reagent solution needed for the luciferin/luciferase reaction
- Add d-luciferin reagent solution to lysed bacteria
- Transform new plates of luciferase with LB on LB/chlor/amp plates

Procedures:

- See the document "Luciferase Expression Protocol"
- Transformations:

- 15 transformations were done in total with the plasmids; 2D (red luciferase), 2F (Epic luciferase), 4J (Orange luciferase), 2H (green luciferase), 2J (yellow luciferase)
- Three trials were done for each transformation with the following amounts of arabinose used for plating:
  - 5mg of arabinose per plate, 6 mg of arabinose per plate, 7 mg of arabinose per plate

Results:

- The transformations look like they're on steroids, i.e. there is way too much growth on all of the plates
- When d-luciferin was added to the luciferase we finally observed a faint glow! (although it was only for yellow luciferase)

August 18th

Objectives:

- Autoclaved inoculation tubes and pipette tips in the morning
- Inoculate

Procedures:

- Inoculations:

Plasmid:	Amount of LB:	Amount of Chloramphenicol:	Amount of arabinose:	Concentration of arabinose:
yellow 1(taken from 5 mg arabinose plate)	3mL	3uL	15 uL	100 mg/mL
yellow 2 (taken from 6 mg arabinose plate)	3mL	3uL	15 uL	100 mg/mL
yellow 3 (taken from 7 mg arabinose plate)	3mL	3uL	15 uL	100 mg/mL
yellow control	3mL	3uL	None	None
orange 1(taken from 5 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
orange 2 (taken from 7 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
orange control	3mL	3uL	None	None
Epic 1 (taken from 5 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
Epic 2 (taken from 7 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
Epic Control	3mL	3uL	None	None
Green 1(taken from	3mL	3uL	15 uL	100 mg/mL

5 mg ara plate)				
Green 2 (taken from 7 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
Green Control	3mL	3uL	None	None
Red 1 (taken from 5 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
Red 2 (taken from 7 mg ara plate)	3mL	3uL	15 uL	100 mg/mL
Red Control	3mL	3uL	None	None

Results:

- After ~18 hours of inoculation, all tubes have transformed culture

August 19th:

Objectives:

- Lyse all of the transformed luciferases from August 18th
- Add the luciferin solution to the lysed luciferases and observe glow
- Transform XJTU bacteria
- Inoculate more yellow luciferase plasmids

Procedures:

- Since each luciferase had ~3 mL of culture and our protocol calls for 350 uL of STET Buffer per each 1 - 2 mL of inoculated cell culture, I used about 700 uL of STET and 50 uL of lysozyme for each inoculation
- The bacteria were lysed at room temperature for two hours and then the lysis was extracted and transferred to a new tube
- The luciferin was added (I did not have the time to experiment with how much luciferin solution needed to be added, so our procedure is not yet optimized)
- XJTU gave us their yellow and blue chromoproteins plasmids pre-expressed in bacteria
    - All we had to do for transformation was our normal transformation protocol on LB and ampicillin plates

Results:

- Unfortunately, only the yellow luciferase lysates exhibited light
    - I decided to inoculate more yellow luciferase plasmids to get more pictures for our wiki and also to show Professor Kang what we've done
- After transformation, the XJTU chromoproteins didn't seem to express any color
    - According to them, they were only able to see color after inoculation and the addition of IPTG

August 20th:

Objectives:

- Lyse inoculated yellow luciferase and add luciferin/ATP mixture

- Inoculate XJTU chromoproteins

Procedures:

- I followed the “Luciferase Expression Protocol” - the bacteria was allowed to lyse for 2 hours at room temperature and then luciferin was added in a dark room
- The XJTU chromoproteins were inoculated first thing in the morning with 5 mL of LB and 5 uL of ampicillin
    - Once the OD600 reached 0.5, 5uL of IPTG was added to each inoculation

Results:

- After overnight inoculation, the XJTU chromoproteins showed color!
- All of the yellow luciferases emitted light except for the control (in which no arabinose was added to the inoculation tube)
    - We discovered that adding larger amounts of luciferin didn’t make the light brighter but increased the duration of the light
    - Professor Kang saw this process and noted that we should either lyse the bacteria for longer or sonicate the cells to get a more intense light